Comm.
Dr. Loosli
Dr. Sommers
Dr. Wyatt

CHRONIC PULMONARY DISEASES

No. 916

RESEARCH PROPOSAL

"IMMUNOLOGIC FUNCTIONS OF HUMAN ALVEOLAR MACROPHAGES"

Robert A. Goldstein, M.D.

Bernard W. Janicki, Ph.D.

TABLE OF CONTENTS

CHRONIC PULMONARY DISEASES

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 50TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: 6/25/73

1. Principal Investigator (give title and degrees):

Robert A. Goldstein, M.D., Chest Physician, Veterans Administration Hospital, Washington, D.C.
Bernard W. Janicki, Ph.D., Chief, Pulmonary Immunology Research Laboratory, Veterans Administration Hospital, Washington, D.C.

2. Institution & address:

Medical Research Associates, Inc.
Suite 511, 1133 Fifteenth Street, N.W.
Washington, D.C. 20005

3. Department(s) where research will be done or collaboration provided:

Chest Section, Medical Service and Pulmonary Immunology Res. Lab.
Veterans Administration Hospital
50 Irving Street, N.W.
Washington, D.C. 20422

4. Short title of study:

Immunologic Functions of Human Alveolar Macrophages

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 3 years

7. Brief description of specific research aims:

The overall objective of this project is to gain insight into the role of the lung as an immunologic organ by critically examining selected biologic functions of the human alveolar macrophage. Furthermore, an attempt will be made to examine the influence of certain disease processes and environmental factors on these functions.

The specific aim of this project is to describe the immune reactivity of alveolar macrophages in patients with pulmonary infections (acute and chronic), in patients with obstructive lung disease (bronchitis and emphysema), and in patients with lung cancer. We also aim to define the qualitative and quantitative effects of cigarette smoking, the effects of extrapulmonary diseases (diabetes, cirrhosis, alcoholism, etc.), and the effects of therapy and environmental alterations on the functional capacity of alveolar macrophages.

8. STATEMENT OF WORKING HYPOTHESIS

A. Background

In recent years, studies in experimental animals have lent considerable support to the concept that one of the most important nonrespiratory functions of the lung is its role in both the establishment and maintenance of immunity. The concept of local lung antituberculosis immunity developed by Dannenberg (1) has been supported recently by Barclay et al. (2) who have shown that rhesus monkeys immunized by means of aerosolized BCG achieved protection against a subsequent challenge with virulent tubercle bacilli without, at the same time, manifesting significant delayed skin hypersensitivity to PPD. Barbaras and Willoughby (3) have shown that rabbits immunized by aerosolized bovine serum albumin were able not only to manifest systemic humoral responses but also developed pathology in the lung similar to that experienced by persons with extrinsic allergic alveolitis.

Moreover, other investigators have shown that alveolar macrophages play an important role in antigenic processing by the lung and also constitute a functionally unique cell population when compared with other macrophages in the reticuloendothelial organs of the body. Leake et al. (4) showed that alveolar macrophages and peritoneal macrophages of rabbits were constitutively different in their enzymatic activities. Holub and Hauser (5) found that intrapulmonary instillation of sheep red blood cells into rabbits produced systemic humoral immunity by eliciting primarily an alveolar macrophage response with very little involvement of mediastinal lymph nodes. Ford and Kuhn (6,7) demonstrated that, following intranasal immunization with sheep red blood cells, rabbit alveolar macrophages were capable of synthesizing immunoglobulins and concluded that these cells were an important source of some of the immunoglobulins in respiratory secretions.

We conclude that the immunologic functions of the lung are more important than heretofore recognized and that the alveolar macrophages comprise one of the most important cell populations in mediating immune responses by the lung.

Attempts to extend such studies to humans have been limited by the unavailability of a viable alveolar cell population to study. Recently however, the widespread use of fiberoptic bronchoscopy in the diagnosis and treatment of pulmonary disorders has permitted for the first time examination of cell populations obtained from the most peripheral portions of the human lung. The technique of bronchopulmonary lavage, which had been previously utilized in the treatment of persons with alveolar proteinosis, status asthmaticus and cystic fibrosis (8,9), has been utilized for limited studies in healthy persons and has demonstrated the feasibility of this technique for the recovery of viable alveolar macrophages (10,11). To date, however, few studies of the immune function of these cells are available even though preliminary data suggest that techniques developed from the study of alveolar

macrophage function in animals can be applied in humans (12).

B. Rationale

It has been suggested that cigarette smoking is not only of specific etiologic significance but also exerts a major deleterious influence in a variety of pulmonary diseases, including chronic bronchitis, emphysema and lung cancer (13). Because attempts to change the smoking habits of persons with these diseases have been largely unsuccessful, it seems imperative to develop specific information about the relationship between cigarette smoking and specific biologic functions of the lung in order to provide meaningful data relevant to reducing the purported health hazards of smoking.

The influence of smoking has been examined in animal and human experiments by a variety of morphologic, physiologic and biochemical parameters. Green and Carolin (14) showed that cigarette smoke depressed the phagocytic activity of alveolar macrophages in rabbits. Martin (15) showed that alveolar macrophages from otherwise healthy smokers contained an increased quantity of abnormally appearing cytoplasmic inclusions and of lysozomal enzymes. Yeager (16) demonstrated that smoke depressed protein synthesis by rabbit alveolar macrophages. On the other hand, Holt and Keast (17) showed that macrophages exposed to low levels of cigarette smoke for long periods had increased protein and RNA synthesis. Moreover, Harris and co-workers (11) found that in vitro phagocytosis and killing of Staphylococcus aureus by alveolar macrophages from smokers and non-smokers proceeded equally well, although smokers' cells had higher resting energy requirements. Little is known however, about the influence of cigarette smoking upon the immune functions of human alveolar macrophages. Warr and Martin (18), in a preliminary study, concluded that alveolar macrophages from otherwise healthy smokers had lost their ability to recognize and respond to specific antigens. Review of their data reveals that comparative experiments were not actually performed. The increased number of alveolar macrophages which are customarily obtained from smokers was not considered when migration chambers were prepared. Furthermore, in order to achieve statistical significance, a longer than usual period of migration (48-72 hours) was permitted. Collectively, these results suggest that further observations are necessary.

The present study is intended to resolve some of these questions. Sophisticated technology from animal experiments can be applied to the study of immune function of human alveolar macrophages. Differential effects related to underlying pulmonary or extrapulmonary diseases can be quantitated. Comparative knowledge based on quantitative and qualitative aspects of smoking can be achieved. The influence of environmental change (either acute or chronic) and therapy also can be examined.

C. Hypothesis

Based on experimental animal studies and upon very preliminary studies in humans, (10-12, 19-22), it is our hypothesis that the alveolar macrophage plays an important role in the immunologic function of the lung. Appreciation of the lung as an immunologically competent organ has been generally under-emphasized. Although characterization of this function in humans has been impeded by lack of material to study, the widespread clinical use of fiberoptic bronchoscopy and the safety of local lung lavage, have made possible the examination of this function in living subjects. Implications about the adverse effects of cigarette smoking upon this function remain to be critically examined in humans. In addition to the influence of smoking, pulmonary and extrapulmonary disease and environmental alteration must be examined in order to provide meaningful data about pathogenic mechanisms of human disease and potentially specific information required for the counseling of any one individual on the influence of cigarette smoking on his health.

D. Significance

Definition of the number, kind and functional status of cells obtained from local lung lavage in certain disease states when related to qualitative and quantitative aspects of cigarette smoking is expected to provide information that is directly relevant to clinical problems. Despite the importance of the lung as an immunologic organ, human studies, to date, have failed to elucidate the specific influence of cigarette smoking upon this function. For example, it is uncertain that alveolar macrophages obtained from smokers are any more or less capable of responding in an immunologically competent fashion to aerosol immunization or to pulmonary infection. Furthermore, it is not known whether heretofore described deficits attributed to smoke in animal experiments have any biologic relevance in relation to the production of human diseases. It has been suggested, for example, that emphysema, which can be produced in experimental animals by enzymatic destruction of lung tissue (23), is also caused in humans by a similar mechanism. Because alveolar macrophages, especially those obtained from smokers, contain some of these enzymes in increased quantities (15) it has been presupposed that this is of pathogenetic significance.

It also is anticipated that this study will provide a general clarification of the role of the alveolar macrophage in the immunologic function of the lung in relation to pulmonary and extrapulmonary disease, specifically to the influence of cigarette smoking. Many of the patients studied will have other illnesses such as hypertension, cirrhosis, and alcoholism, among others. Thus, if cigarette smoking alters alveolar macrophage function in a very minor way, it will be possible to compare and contrast results obtained from persons with a variety of diseases

in order to elucidate the alterations that such co-existing events may create.

We feel that the present proposal offers an opportunity to extend animal experiments to the more relevant clinical condition, to describe in a detailed fashion the immune function of alveolar macrophages in humans, and to relate derangements in function to specific host and environmental factors.

It is hoped that clear demonstration of specific host or environmental influences and potential alteration by medical therapy or environmental change, may lead to more constructive personal counseling. Persons are generally more receptive to medical recommendations that are of direct personal relevance than to blanket recommendations based on studies of other people or experimental studies in animals.

9. DETAILS OF EXPERIMENTAL DESIGN AND PROCEDURES

A. Study Subjects

1. Source

Subjects will be selected from among those persons in whom diagnostic and/or therapeutic fiberoptic bronchoscopy is clinically indicated. In addition to the standard hospital consent forms, an additional explanation will be made to those persons selected for study and informed consent will be obtained prior to limited lung lavage. A sample of the consent form and a letter from the Human Studies and Research Committees at this institution granting approval for this study are appended as attachments 1 and 2 respectively.

A complete history, physical and laboratory examination including routine blood work, skin test series, chest roentgenogram, electrocardiogram and pulmonary function studies (including arterial blood gas analysis) will be performed. A detailed respiratory history and environmental exposure will be recorded utilizing the standard questionaire developed for this purpose by the British Medical Research Council (see attachment 3). Cigarette consumption, both qualitative (filter and non-filter) and quantitative (daily amount and years exposure) will be recorded.

2. Types of Disease States

At the outset, only persons with chronic lung disease (bronchitis and emphysema) pulmonary infections (pneumonia and lung abscess) and lung cancer will be examined. Patients will be studied initially at a time when fiberoptic bronchoscopy appears to be diagnostically or therapeutically indicated. Subsequently, specific and controlled environmental and therapeutic changes will be made under closely supervised conditions in a Respiratory Care Unit. Thus, cessation of smoking will be enforced

both for therapeutic and study purposes in this manner. Repeat studies will be performed usually within 2 to 3 weeks. Practically speaking, this is an appropriate time interval to establish optimum pulmonary toilet, antibiotic therapy, and other forms of medical care. In persons with lung cancer, fiberoptic bronchoscopy is often the initial diagnostic procedure and leads to subsequent evaluation regarding operability. This "lag" period usually extends 1-2 weeks; it is anticipated that a repeat study in these persons can be performed just prior to the time of surgery. In this manner, each person is expected to serve as his own control. Serial studies in the same person serve to keep constant as many human variables as possible. In contrast, single studies in a large group of persons are less satisfactory because of extreme biologic variations. No studies are anticipated at this time in healthy persons. There are two reasons for this, neither related to the potential scientific merits of such examinations. In the first place, the Human Studies Committee at this institution suggested (not insisted), that caution at the initiation of such a study appeared to be in the best interests of the Hospital and the patient. Since potential information of therapeutic value might be achieved in persons who would otherwise be bronchoscoped, the initial procedure could be of direct benefit to the patient and not be construed as a procedure performed for "research purposes" only. In the second place, the Committee felt that a decision should be made concerning the study of healthy persons in whom there would be no clinical indication for the procedure after sufficient information was obtained and evaluated in a patient population.

3. Bronchoscopy and Lung Lavage

Following an overnight fast, the subject is seated in a dental chair. Local anesthesia will be accomplished by application of viscous and liquid 1% xylocaine to the nasopharynx and vocal cords. The distal end of the fiberoptic bronchoscope will be passed under direct vision into the trachea. Following the indicated diagnostic evaluation and using an amount of local anesthetic not to exceed 250 to 300 mg xylocaine (as a 1% solution to a volume usually of 30 ml), the bronchoscope will be passed into the right or left lower lobe. A segmental local lavage, restricted to one area, then will be performed using 50 ml aliquots of sterile, pyrogen-free 0.9% saline which has previously been warmed to 37°C (24). Fluid will be allowed to flow in by gravity and removed by gentle suction with a syringe. It is anticipated that approximately one-half of each aliquot will be recovered and that no more than 6 aliquots will be utilized. Until the cell yield can be determined per aliquot in various disease states, we will initiate the series of experiments with only one 50 ml local lavage. Presumably following an initial series of studies, the actual number of aliquots necessary to achieve the desired cell numbers can be determined. In either instance we shall strive to use the least possible number and in no case exceed a

total of 6 (or 300 ml). Following the procedure, patients will be observed in the usual manner; that is, no oral intake until the gag reflex has returned, and follow-up physical, laboratory and x-ray examinations will be performed as indicated. It is anticipated that the lavage will add approximately 15 minutes to the overall bronchoscopic examination.

As recommended by the manufacturer, the flexible tube of the bronchoscope will be sterilized by immersion in 30% ethanol overnight. After soaking, it will be wiped with a gauze sponge soaked in alcohol and subsequently dried with a sterile sponge and stored in a sterile cloth bag. The tube will be handled using sterile gloves while the lens cleanser and xylocaine jelly are applied prior to insertion. After each use, the flexible tube will be wiped with a gauze sponge soaked in 30% alcohol and the aspiration channel will be rinsed and brushed. These precautions are considered reasonable and are in keeping with attempts not to destroy the delicate optical fibers by more rigorous sterilization processes such as autoclaving and/or gas sterilization, which have been found to be harmful to the scope. Periodic cultures will be made to determine the effectiveness of the sterilization procedure.

4. Immunologic Profile

a. In Vivo Tests

Intradermal reactivity to a battery of test antigens including tuberculin PPD, streptokinase, Candida albicans, and mumps, will be determined in all study subjects. The skin reactions will be measured and recorded 48 hours after injection.

b. In Vitro Tests

To determine in vitro cellular immune responses, a heparinized venous blood sample will be drawn from each study subject. Leukocyte-rich plasma (LRP) will be obtained after erythrocyte sedimentation on standing at room temperature for 1 to 2 hours. The LRP will be processed for study as described below and the sedimented blood will be centrifuged at 2,100 g for 20 minutes at 5 C to obtain platelet-depleted, autologous plasma.

1) Lymphocyte Blastogenesis

The cells of the LRP will be sedimented by centrifugation and resuspended to contain approximately 1 x 10^6 lymphocytes in 3 ml of culture medium containing 80% medium 199 and 20% autologous, cell-free plasma, as described previously (25). The response of the cultured cells to selected specific antigens and to non-specific mitogenic stimuli, such as phytohemagglutinin, concanavalin A, and pokeweed mitogen, will be examined. Triplicate 3 ml cultures will be prepared for each

stimulus; cultures to which no stimulus was added will be used as negative controls. After an appropriate period of incubation at 37 C, each culture will be subjected to a 3 hour pulse with 0.03 µCi of ^{3}H thymidine. The cultures then will be processed (26) for assay of radioactive trichloroacetic-acid precipitable material. Lymphocyte blastogenesis will be expressed as an S/C ratio which is calculated by dividing the radioactivity (DPM) of the cultures to which the stimulus was added (S) by that of the unstimulated negative control (C) cultures.

2) Lymphokine Production

The release of migration inhibitory factor (MIF) into the medium on culture of blood lymphocytes with selected, specific, antigens will be used as an indicator of lymphokine production. The methods described by Rocklin and his coworkers (27) will be used for this purpose. Blood lymphocytes in the LRP will be purified by passage through a nylon-cotton column and subsequently cultured for 4 days at 37 C in medium with and without specific antigens. The culture medium will be changed on a daily basis; the cell-free supernatant culture fluids, collected daily, will be appropriately pooled, dialyzed, and concentrated by freeze-drying. MIF activity will be assayed routinely using oil-induced peritoneal exudate cells from normal guinea pigs by methods developed previously in this laboratory (28,29). Occasionally, as described below, a preparation with confirmed MIF activity will be used to examine the functional status of human alveolar macrophages.

B. Profile Studies of Alveolar Cells

The analysis of alveolar macrophage immunologic reactivity necessitates an assessment of their functional state. For this reason, the alveolar cells will be examined by various parameters, as described below, to develop a functional profile.

1. Cell Isolation Techniques

The pooled alveolar washings will be processed aseptically for study on the day of collection. For morphologic characterization of the cell suspension, the cells contained in a 5 ml aliquot of the pooled washings will be isolated by membrane filtration (5µ porosity filter). The cells on the filter will be washed once with an equal volume of saline and once with 15 ml of 95% ethanol after which the wet filter pad will be transferred to a staining dish for subsequent processing. The remainder of the pooled washings will be centrifuged at 300g for 10 minutes at 4 C to sediment the cells. The packed cells will be washed twice by centrifugation using Hanks balanced salt solution (BSS) and

finally resuspended in a minimal volume of BSS to provide an approximate 100-fold concentration. The cell populations in the final suspension will be enumerated by total and differential counts; macrophage viability will be determined by the eosin Y-exclusion test described by Hanks and Wallace (30).

2. Morphologic and Histochemical Characterization

a. Morphology

The alveolar cell preparation will be processed for morphologic examination by light microscopy. For this purpose, the cells isolated on the membrane filter will be fixed with 95% ethanol and stained with hematoxylin and eosin. The stained preparation will be dehydrated progressively with ethanol, clarified with xylene, and mounted on glass slides. As described by Martin (15), the stained cells will be characterized on the basis of cytoplasmic inclusions, number of nuclei per cell, and cell diameter.

b. Histochemistry

The enzymatic capabilities of alveolar macrophages will be characterized qualitatively by histochemical techniques. As suggested by Dannenberg (1), an estimate of macrophage "activation" can be obtained from histochemical analyses of representative lysozomal and mitochondrial enzymes. For this purpose, the methods developed by Dannenberg and his associates (31) for study of rabbit alveolar macrophages will be modified and adapted. The concentrated alveolar cell suspension will be used to prepare smears on Mylar film or glass slides which will be air dried and stored in the cold for subsequent study. The β-galactosidase and cytochrome oxidase reactivity of the alveolar macrophage will be examined as representative lysozomal and mitochondrial enzymes, respectively, using the fixatives and substrates described by Dannenberg and his associates (31,32). As indicated for individual patients in the study, other macrophage enzymes will be examined using similar methodology. Also, as indicated, selected enzymes can be assayed quantitatively in lysates of alveolar macrophages using the methods described by others for human (15) and animal (4, 31-33) cells. It is planned that our capability in these techniques will be developed in collaboration with Arthur M. Dannenberg, Jr., M.D. of the Johns Hopkins University School of Medicine; confirmation of this arrangement is provided in the attached letter (Attachment 4).

3. Functional Characterization

a. Phagocytic Activity

The methods described by Massaro and his

coworkers (34) to measure phagocytosis of polystyrene latex beads by rabbit alveolar macrophages will be modified and adapted for the present study. It is planned that approximately 15 x 10^6 macrophages from the concentrated alveolar cell suspension will be incubated with the beads at 37 C for 30 minutes. A 10% suspension of beads will be prepared as they described. Phagocytosis of the beads will be measured by counting the number of macrophages containing beads, and the number of beads/cell, in randomly-selected fields at a 400X magnification. Approximately 200 cells will be examined from each preparation; the results will be expressed as a percentage of phagocytizing macrophages and as a percentage of cells containing 10 or more beads/cell, as suggested previously (34). As required for a more precise measure of phagocytosis of the beads, the quantitative assay technique described by Weisman and Korn (35) will be employed. For this determination, the alveolar macrophages will be sedimented by centrifugation at the end of the incubation period, washed to remove extracellular beads and extracted with dioxane to solubilize the phagocytized beads. The absorbance of the dioxane extract at 259 mμ will be used to provide a quantitative measure of latex contained in the macrophage preparations.

b. Metabolic Activity

Oxygen consumption will be measured manometrically in a Gilson differential respirometer as a representative parameter of the alveolar macrophages' metabolic activity. An aliquot of the concentrated alveolar cell suspension will be incubated in Krebs-Ringer-phosphate solution which contains glucose as the metabolic substrate. The procedures for preparation of solutions, operation of the instrument, and calculations of data will be the same as those described by Umbreit and his collaborators (36); oxygen consumption will be expressed as μl O_2/hr/10^6 cells.

4. Immunologic Reactivity

a. Direct Migration Test

Specific antigen - induced inhibition of macrophage migration, and accepted in vitro correlate of delayed-type hypersensitivity (37), will be employed as a measure of alveolar macrophage immunologic reactivity. The test antigens will be the same as those used in the in vivo tests to develop the study subject's immunologic profile, as described above. The methods used in this laboratory (28) for study of guinea pig macrophage migration inhibition will be modified and adapted for the present study. The concentrated alveolar cell suspension will be supplemented, as required, with leukocyte-rich plasma isolated from the subject's peripheral blood as described above to provide a 5 to 10% lymphocyte concentration. Non-heparinized

capillary tubes will be filled with the suspension, sealed at one end with paraffin wax, and centrifuged. The tubes then will be cut just below the cell-fluid boundary and the sections containing the packed cells will be placed in sterile silicone grease on the bottom of a tissue culture dish. Each dish will contain 4 to 6 capillary tubes and will receive 4 ml of culture fluid. In initial experiments, the culture fluid will contain Eagle's minimal essential medium, 15% autologous serum, 1% L-glutamine, penicillin G (100 units/ml) and streptomycin (0.1 mg/ml); the composition of the culture fluid may be modified, as required, for later experiments to provide an optimal environment for the macrophages. The dishes will be sealed with transparent tape and incubated at 37 C for 24 hours after which migration of cells from the capillary tip will be recorded by tracing a 15.6X projection of the area surrounding the capillary tip. The area of cell migration will be measured by planimetry of the tracing. Macrophage migration in the presence of the test antigens will be expressed as a percentage of the migration area of the same cell suspensions in medium without the test antigens. At least 4 capillaries each for control and test antigen cultures will be measured for each suspension.

b. Response to Lymphokine

An indirect migration inhibition test also will be used to examine the immunologic reactivity of alveolar macrophages. In this test, the response of alveolar macrophages to migration inhibitory factor (MIF) will be evaluated. The MIF lymphokine will be produced by incubating column-purified peripheral blood lymphocytes from a tuberculin hypersensitive donor with PPD using the methods described above. Capillary tubes containing the study subject's alveolar macrophages will be cultured both in a control and MIF-containing medium. Measurement of migration areas and calculation of migration inhibition by MIF will be as described above for the direct migration test.

5. Statistical Analysis

At the outset, no healthy subjects will be studied to provide "control data". It is anticpated, however, that internal group comparisons of persons with a variety of diseases can be made at a later date. Thus, similar parameters can be compared in subjects with the same diseases at different times by a paired t test (38).

6. Timing of Studies

It is anticipated that the early phases of this project will be devoted to the modification and adaptation of

existing methodology from animal models for study of human alveolar macrophages. This will necessitate preliminary studies in humans to obtain baseline information concerning cell yield and other technical aspects of the experiments. As indicated earlier, the assistance to be provided by Dr. Dannenberg should facilitate development of our capability for histochemical methodology. Dr. Donald J. Massaro, Chief of the Pulmonary Disease Section at this hospital, will provide similar assistance in adapting methods for study of the phagocytic and metabolic activities of human alveolar macrophages. Our prior experience with methods for study of cell-mediated immune phenomena in related human and animal models will be employed to finalize the tissue culture methods for migration studies of human alveolar macrophages. This early phase should require no more than 3-4 months, following which we shall be able to perform complete functional profiles in selected patients. Subsequent to that time it is planned that 2 complete studies will be attempted each week, in anticipation that at least one complete set of results will be obtained weekly. On this basis, we project that approximately 64 complete studies will be performed during the first year. It is anticipated that once an adequate number of experiments has been performed in patients with the aforementioned disease states, the protocol will be expanded for the study of healthy volunteer subjects. The latter group should provide a more complete evaluation of alveolar macrophage function. Since the study of healthy volunteers will require direct financial compensation for each subject, we anticipate an additional request in subsequent budgets for this purpose. We expect to complete the entire series of experiments within 3 years.

C. Literature Cited

1. Dannenberg, A.M., Jr. Cellular hypersensitivity and cellular immunity in the pathogenesis of tuberculosis: Specificity, systemic and local nature, and associated macrophage enzymes. Bact. Revs., 32:85-102, 1968.

2. Barclay, W.T., Busey, W.M., Dalgard, D.W., Good, R.C., Janicki, B.W., Kasik, J.E., Ribi, E., Ulrich, C.E., and Wolinsky, E. Protection of monkeys against airborne tuberculosis by aerosol vaccination with Bacillus Calmette-Guerin. Amer. Rev. Resp. Dis., 107:351-358, 1973.

3. Barbaras, J.E. and Willoughby, W.F. Immunopathologic studies of antigen inhalation. Amer. Rev. Resp. Dis., in press, 1973.

4. Leake, E.S., Gonzalez-Ojeda, D., and Myrvik, Q.N. Enzymatic differences between normal alveolar macrophages and oil-induced peritoneal macrophages obtained from rabbits. Exptl. Cell. Res., 33: 553-561, 1964.

5. Holub, M. and Hauser, R.E. Lung alveolar histiocytes engaged in antibody production. Immunol., 17: 207-226, 1969.

6. Ford, R.J., Jr. and Kuhn, C. Immunologic competence of alveolar cells. I. The plaque-forming response to particulate and soluble antigens. Amer. Rev. Resp. Dis., 107:763-770, 1973.

7. Ford, R.J., Jr. and Kuhn, C. Immunologic competence of alveolar cells. II. Modification of the plaque-forming response by inhibitors, tolerance, and chronic stimulation. Amer. Rev. Resp. Dis., 107: 771-775, 1973.

8. Rogers, R.M. and Tantum, K.R. Bronchopulmonary lavage: a "new" approach to old problems. Med. Clin. N. Amer., 54:755-771, 1970.

9. Kylstra, J.A., Rausch, D.C., Hall, K.D. and Spock, A. Volume-controlled lung lavage in the treatment of asthma, bronchiectasis, and mucoviscidosis. Amer. Rev. Resp. Dis., 103:651-655, 1971.

10. Cohen, A.B. and Cline, J.J. The human alveolar macrophage: Isolation, cultivation in vitro and studies of morphologic and functional characteristics. J. Clin. Invest., 50:1390-1398, 1971.

11. Harris, J.O., Swenson, E.W. and Johnson, J.E., III. Human alveolar macrophages: Comparison of phagocytic ability, glucose utilization, and ultrastructure in smokers and non-smokers. J. Clin. Invest., 49: 2086-2096, 1970.

12. Jurgeson, P.F., Olsen, G.N., Swenson, E.W., Johnson, J.E., Waldham, R.H. and Henney, C.S. Cell mediated immunity to tuberculin demonstrated by human broncho-alveolar lymphocytes. Amer. Rev. Resp. Dis., 105: 955, 1972.

13. U.S. Public Health Service: Smoking and Health: Report of the Advisory Committee to the Surgeon General of the United States, U.S. Dept. of HEW, PHS Publication, No. 1103, Wash., D.C., 1964.

14. Green, G.M. and Carolin, D. The depressant effect of cigarette smoke on the in vitro antibacterial activity of alveolar macrophages. New Eng. J. Med., 276:421-427, 1967.

15. Martin, R.R. Altered morphology and increased acid hydrolase content of pulmonary macrophages from cigarette smokers. Amer. Rev. Resp. Dis., 107: 596-601, 1973.

16. Yeager, H., Jr. Alveolar cells: Depressant effect of cigarette smoke on protein synthesis. Proc. Soc. Exptl. Biol. Med., 131:247-250, 1969.

17. Holt, P.G. and Keast, D. The effect of tobacco smoke on protein synthesis in macrophages. Proc. Soc. Exptl. Biol. Med., 142:1243-1247, 1973.

18. Warr, G. and Martin, R. Effect of smoking on human alveolar macrophage responsiveness to migration inhibition factor. Clin. Res., 21:73, 1973.

19. Green, G. In defense of the lung. Amer. Rev. Resp. Dis., 102:691-703, 1970.

20. Myrvik, Q.N. and Evans, D.G. Metabolic and immunologic activities of alveolar macrophages. Arch. Environ. Health, 14:92-96, 1967.

21. Dumonde, D.C. The role of the macrophage in delayed hypersensitivity. Brit. Med. Bull., 23:9-14, 1967.

22. Cohn, Z.A. The structure and function of monocytes and macrophages. Adv. Immun., 9:163-214, 1968.

23. Marco, V., Mass, B., Meranze, D.R., Weinbaum, G. and Kimbel, P. Induction of experimental emphysema in dogs using leukocyte homogenates. Amer. Rev. Resp. Dis., 104:595-598, 1971.

24. Brain, J.D. and Frank, R. Alveolar macrophage adhesion: wash electrolyte composition and free cell yield. J. Appl. Physiol., 43:75-80, 1973.

25. Janicki, B.W., Aron, S.A., Schechter, G.P. and McFarland, W. Tuberculin antigens active in human lymphocyte blastogenesis. Proc. Soc. Exptl. Biol. Med., 141:809-813, 1972.

26. Moorhead, J.R., Connolly, J.J. and McFarland, W. Factors affecting the reactivity of human lymphocytes in vitro. I. Cell number, duration of culture and surface area. J. Immunol., 99:413-419, 1967.

27. Rocklin, R.E., Meyers, O.L. and David, J.R. An in vitro assay for cellular hypersensitivity in man. J. Immunol., 104:95-102, 1970.

28. Janicki, B.W., Schechter, G.P. and Schultz, K.E. Cellular reactivity to tuberculin in immune and serologically-unresponsive guinea pigs. J. Immunol., 105:527-530, 1970.

29. Goldstein, R.A., Janicki, B.W. and Schultz, K.E. Reactivity of lymphocytes from sarcoidosis patients to Kveim antigen in vitro. Clin. Res., 20:510, 1972.

30. Hanks, J.H. and Wallace, J.H. Determination of cell viability. Proc. Soc. Exptl. Biol. Med., 98:188-192, 1958.

31. Dannenberg, A.M., Jr., Burstone, M.S., Walter, P.C. and Kinsley, J.W. A histochemical study of phagocytic and enzymatic functions of rabbit mononuclear and polymorphonuclear exudate cells and alveolar macrophages. I. Survey and quantitation of enzymes and states of cellular activation. J. Cell. Biol., 17:465-486, 1963.

32. Yarborough, D.J., Meyer, O.T., Dannenberg, A.M., Jr. and Pearson, B. Histochemistry of macrophage hydrolases. III. Studies on β-galactosidase, β-glucoronidase and aminopeptidase with indolyl and naphthyl substrates. J. Reticuloendothel. Soc., 4:390-408, 1967.

33. Axline, S.G. Isozymes of acid phosphatase in normal and Calmette-Guerin bacillus-induced rabbit alveolar macrophages. J. Exptl. Med., 128:1031-1048, 1968.

34. Massaro, D., Kelleher, K., Massaro, G. and Yeager, H., Jr. Alveolar macrophages: depression of protein synthesis during phagocytosis. Amer. J. Physiol., 218:1533-1539, 1970.

35. Weisman, R.A. and Korn, E.D. Phagocytosis of latex beads by Acanthamoeba. I. Biochemical properties. Biochem., 6:485-497, 1967.

36. Umbreit, W.W., Burris, R.H. and Stauffer, J.F. Manometric Techniques, 4th ed., Burgess Publishing Co., Minneapolis, Minn., 1964.

37. Bloom, B.R., Landy, M. and Lawrence, H.S. In vitro methods in cell-mediated immunity: a progress report. Cell. Immunol., 6:331-347, 1973.

38. Snedecor, G.W. and Cochran, W.G. Statistical Methods, 6th ed., The Iowa State Univ. Press., Ames, Iowa, 1968.

Washington Veterans Administration Hospital (WVAH)
George Washington University Medical Center (GWUMC)

Consent for Research

1. I, _____, hereby agree to participate in a program of studies under the supervision of Dr. Robert Goldstein. I understand that while the program will be under the supervision of Dr. Robert Goldstein, other professional persons who work with him may be designated to assist him or to act for him.

2. The studies have been described and their purpose explained to me by Dr. Robert Goldstein. I understand that the studies will involve or require some or all of the following tests or procedures: in addition to having a tube passed through my nose (bronchoscope) and into my lungs, from 50 to 300 ml (as little as 2 ounces or as much as 10 ounces) of fluid will be allowed to go into my lung (segmental lung lavage) and subsequently be removed.

3. I understand that the study includes the following risks: that some of the fluid may remain in my lungs for as much as one or two days following the procedure, and that it is possible I may experience some increased difficulty breathing following the procedure.

I voluntarily accept the risks of these studies. I further understand that every precaution has been and will be taken to remove and reduce risk.

4. I authorize WVAH/GWUMC to keep, preserve, use or dispose of the results of the studies in which I have agreed to participate.

5. I understand that my participation in the study may be terminated upon my wishes or at the discretion of Dr. Robert Goldstein.

_____ _____
Date Signature

I, the undersigned, have defined and fully explained the studies involved to the above.

_____ _____
Date Investigator's Signature

I, the undersigned, having no special interest in the research proposal, and having heard the explanation given by Dr. _____ to _____ certify that the explanation seemed complete and was apparently understood by the patient/subject and that he/she signed this form in my presence.

_____ _____
Date Witness Signature

1003539598

ATTACHMENT #2

REPORT OF SUBCOMMITTEE ON HUMAN STUDIES

TITLE DESCRIPTIVE OF PROPOSAL, APPLICATION, PROCEDURE, DRUG USAGE, STUDY, ETC.
"Immunologic functions of human alveolar cells"

NAME OF PRINCIPAL INVESTIGATOR	NAME AND LOCATION OF VA INSTALLATION
Robert A. Goldstein, M.D.	VAH, Washington, D.C.

1. This is to certify that the grant application entitled "Immunologic functions of human alveolar cells" *(Proposal, application, procedure, drug usage, study)*

identified above has been reviewed by the Research and Education Subcommittee on Human Studies appointed to consider clinical research proposals and other investigations involving human beings.

2. The Subcommittee on Human Studies has considered specifically:

(a) the rights and welfare of the individual(s) involved,
(b) the appropriateness of the methods to be used to secure informed consent, and
(c) the risks and potential medical benefits of the investigation.

3. The collective judgment of the Subcommittee is that: *(Check (a) or (b))*

☒ (a) the proposed drug usages and/or procedures are considered appropriate, and approval is recommended.

☐ (b) the proposed drug usages and/or procedures are considered inappropriate, as presented, and approval is not recommended for the reasons stated in the attached memorandum.

4. This proposal is consistent with the policy set forth in Chapter 8, DM&S Manual M-2, Part XIV.

100339599

DATE OF SUBCOMMITTEE ACTION	SIGNATURE (of the Subcommittee on Human Studies)
4/9/73	WILLIAM McFARLAND, M.D.

FINAL ACTION	DATE SIGNED	SIGNATURE OF CHAIRMAN OF RESEARCH AND EDUCATION COMMITTEE
☒ APPROVED ☐ DISAPPROVED	5/3/73	B.W. JANICKI, Ph.D.

VA FORM APR 1967 10-1223

440558

ATTACHMENT #3

APPENDIX A

Following is the questionnaire used in the study, essentially the one approved in 1966 by the British Medical Research Council's Committee on Research into Chronic Bronchitis (modifications consisted of substitutions of U.S. for English idiom, e.g., "mucus" for "phlegm", and the addition of two preliminary data, age and sitting height).

QUESTIONNAIRE ON RESPIRATORY SYMPTOMS

SURVEY OR HOSPITAL NUMBER..... Date of interview_____

Date of birth_____

NAME..... Age_____

(Surname) Sex_____

..... Civil state_____

(First names) Standing height (inches)_____

..... Sitting height (inches)_____

ADDRESS..... Weight (lbs.)_____

..... Occupation_____

..... Race_____

..... Name of interviewer_____

Use the actual wording of each question. Put X in appropriate square after each question. When in doubt record 'No'.

PREAMBLE I am going to ask you some questions, mainly about your chest. I would like you to answer 'YES' or 'NO' whenever possible.

COUGH

1. Do you usually cough first thing in the morning (on getting up*) in bad weather?

☐ Yes ☐ No

Count a cough with first smoke or on first going out of doors. Exclude clearing throat or a single cough.

*For subjects who work by night.

Page 2

3. Do you usually cough during the day--or at night--in bad weather? ☐ Yes ☐ No

Ignore an occasional cough.

If 'No' to both questions 1 and 3, go to question 6.

If 'Yes' to either question 1 or 3:

5. Do you cough like this on most days (or nights*) for as much as three months each year? ☐ Yes ☐ No ☐ N.A.

MUCUS

6. Do you usually bring up any mucus from your chest first thing in the morning (on getting up*) in bad weather? ☐ Yes ☐ No

Count mucus with the first smoke or on first going out of doors. Exclude mucus from the nose. Count swallowed mucus.

8. Do you usually bring up any mucus from your chest during the day--or at night--in bad weather? ☐ Yes ☐ No

Accept twice or more.

If 'No' to both questions 6 and 8, go to question 12a.

If 'Yes' to either question 6 or 8:

10. Do you bring up mucus like this on most days (or nights*) for as much as three months each year? ☐ Yes ☐ No ☐ N.A.

12a. In the past three years have you had a period of (increased**) cough and mucus lasting for three weeks or more? ☐ No

If 'No' to question 12a, go to question 13.

If 'Yes' to question 12a: ☐ Yes--1 period

12b/c. Have you had more than one such period? ☐ Yes--2 or more periods

*For subjects who work by night.

**For subjects who usually have mucus.

13. Have you ever coughed up blood? ☐ No

If 'No' to question 13, go to question 14a.

If 'Yes' to question 13: ☐ Yes--in past year

13a. Was this in the past year? ☐ Yes--not in past year

10035396O1

Page 3

BREATHLESSNESS

14a. Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? Disabled* ☐

If 'No' to question 14a, go to question 15a. No--a ☐

If 'Yes' to question 14a:

14b. Do you get short of breath walking with other people of your own age on level ground? No--b ☐

If 'No' to question 14b, go to question 15a.

If 'Yes' to question 14b:

14c. Do you have to stop for breath when walking at your own pace on level ground? No--c ☐

*Disabled from walking by any conditions other than heart or lung disease. Yes--c ☐

WHEEZING

15a. Does your chest ever sound wheezing or whistling? No ☐

If 'No' to question 15a, go to question 16a.

If 'Yes' to question 15a: Yes, but not most days (or nights) ☐

15b. Do you get this most days--or nights? Yes, most days (or nights) ☐

16a. Have you ever had attacks of shortness of breath with wheezing? No attacks ☐

If 'No' to question 16a, go to question 17.

If 'Yes' to question 16a: No ☐

16b. Is/was your breathing absolutely normal between attacks? Yes ☐

WEATHER

17. Does the weather affect your chest?

Only record 'Yes' if adverse weather definitely and regularly causes chest symptoms. No ☐

If 'No' to question 17, go to question 18.

If 'Yes' to question 17: Yes ☐

17a. Does the weather make you short of breath? No ☐

10035396O2

Page 4

17b. Specify type of weather, e.g. fog, damp, cold, heat, other ☐

NASAL CATARRH

18. Do you usually have a stuffy nose or mucus at the back of your nose in the winter? ☐ Yes ☐ No

19. Do you have this in the summer? ☐ Yes ☐ No

If 'No' to both questions 18 and 19, go to question 21.

If 'Yes' to either question 18 or 19:

20. Do you have this on most days for as much as three months each year? ☐ Yes ☐ No ☐ N.

CHEST ILLNESSES

21. During the past three years have you had any chest illness which has kept you from your usual activities for as much as a week? ☐ No

If 'No' to question 21, go to question 22.

If 'Yes' to question 21:

21a. Did you bring up more mucus than usual in any of these illnesses? ☐ No

If 'No' to question 21a, go to question 22.

If 'Yes' to question 21a:

21b. How many illnesses like this have you had in the past three years? ☐ 1 illness ☐ 2 or more illnesses

HAVE YOU EVER HAD:

22. An injury or operation affecting your chest?* ☐
23. Heart trouble?* ☐
24. Bronchitis?** ☐
25. Pneumonia** ☐
26. Pleurisy?** ☐
27. Pulmonary tuberculosis?* ☐
28. Bronchial asthma?* ☐
29. Emphysema?* ☐
30. Bronchiectasis?* ☐
31. Other chest trouble?** ☐

* Code: 0=no; 1=once; 2=twice.....nine or more times.

** Code 0=no; 1=yes

Give relevant details after each positive answer.

100353603

Page 5

TOBACCO SMOKING

35a. Do you smoke? ☐ Yes ☐ No

Record "Yes" if regular smoker (as defined in question 35b) up to one month ago.

If 'No' to question 35a, ask question 35b.

If 'Yes' to question 35a:

Do you inhale the smoke? ☐ Yes ☐ No

Would you say you inhale the smoke slightly (S), moderately (M), deeply (D)? ☐ S ☐ M ☐ D

How old were you when you started smoking regularly?

___years old

How many manufactured cigarettes do you usually smoke per day?

___per working day

___at weekends

How much tobacco (oz/g) do you usually smoke per week in hand-rolled cigarettes?

_____

How much pipe tobacco (oz/g) per week were you smoking before you gave up?

_____

How many cigars do you usually smoke per week? _____

Specify large (L) or small (S).

35b. Have you ever smoked as much as one cigarette a day (or one ounce of tobacco a month) for as long as a year? ☐ Yes ☐ No

CODING FOR SMOKING HISTORY

Before coding refer to instructions

Smoking history

- Never smoked ☐
- Ex-smoker ☐
- Present smoker--does not inhale ☐
- Present smoker--inhales slightly ☐
- Present smoker--inhales moderately ☐
- Present smoker--inhales deeply ☐

Type of smoker

- Cigarettes only ☐
- Pipe only ☐
- Cigars only ☐
- Cigarettes and pipe/cigars ☐
- Cigars and pipe ☐
- Non-smoker ☐

Amount smoked per day* (average including weekends)

Cigarette tobacco:

- Nil ☐
- 1-4 g ☐
- 5-14 g ☐
- 15-24 g ☐
- 25 g or more ☐

*1 oz of pipe tobacco=28 cigarettes =28 g

1 small cigar = 2 cigarettes

1 large cigar = 5 cigarettes

100353604

If 'No' to question 35b, go to question 38.

If 'Yes' to question 35b:

How old were you when you started smoking regularly?

___years old

How old were you when you last gave up smoking?

___years old

How many manufactured cigarettes per day were you smoking before you gave up?

___per working day

___at weekends

How much tobacco (oz/g) per week were you smoking in hand-rolled cigarettes before you gave up?

_____

How much pipe tobacco (oz/g) per week were you smoking before you gave up?

_____

How many cigars per week were you smoking before you gave up?

_____

Specify large (L) or small (S).

OCCUPATION

Record on dotted lines number of years in which subject has worked in any of these industries.

38. Have you ever worked in a dusty job?

39. At a coalmine ..... ☐ Yes ☐ No

Pipe/cigar tobacco:

Nil

1-4 g

5-14 g

15-24 g

25 g or more

Age started (years) ☐☐ Code XX if a non-smoker

Age stopped (years) ☐☐ Code YY if a present smoker

Page 7

40. In any other mine..... ☐ Yes ☐ No

41. In a quarry..... ☐ Yes ☐ No

42. In a foundry..... ☐ Yes ☐ No

43. In a pottery..... ☐ Yes ☐ No

44. In a cotton, flax or hemp mill..... ☐ Yes ☐ No

45. With asbestos..... ☐ Yes ☐ No

46. In any other dusty job. ☐ Yes ☐ No

If 'Yes', specify

.....

Total number of years in dusty job?..... ☐☐

47a. Have you been exposed regularly to irritating gas or chemical fumes?

Yes, 47a and b ☐

No, 47a and b ☐

If 'Yes', give details of nature and duration.

..... Yes 47a } ☐

..... No 47b

47b. Have you ever been off work for a shift or longer following acute exposure to gases or fumes? No 47a } ☐ Yes 47b

If 'Yes', give details of nature and duration.

.....

.....

.....

.....

TIME_____AMBIENT TEMPERATURE°C_____

FEV	VITAL CAPACITY	MMEF
3_____ 4_____	3_____ 4_____	3_____ 4_____
5_____	5_____	5_____
☐☐☐	☐☐☐	☐☐☐

FEV %VC ☐☐

10035396

ATTACHMENT #4

THE JOHNS HOPKINS UNIVERSITY

SCHOOL OF HYGIENE AND PUBLIC HEALTH

DEPARTMENT OF RADIOLOGICAL SCIENCE

615 North Wolfe Street • Baltimore, Maryland 21205

June 6, 1973

Dr. Bernard Janicki
Veterans Administration Hospital
50 Irving Street, N.W.
Washington, D.C. 20422

Dear Bernard:

We shall be happy to train your technician in our laboratory in the various histochemical procedures we use, and be of any other assistance that may be required to launch your proposed program on the alveolar macrophages of man.

With very best regards,

Sincerely,

Art

Arthur M. Dannenberg, Jr., M.D.
Associate Professor of
Radiobiology and Pathology

AMD:ms

Enclosures: Reprints

10 Space and facilities available (when elsewhere than item 2 indicates, state location):

All phases of this project will be conducted at the Washington, D.C., Veterans Administration Hospital. The selection of study subjects, as well as the bronchoscopic and lavage procedures, will be under the direct supervision of Robert A. Goldstein, M.D. He has performed and/or supervised fiberoptic bronchoscopy during the course of routine diagnostic and therapeutic evaluations at the VA Hospital since July 1, 1972. A sufficiently varied clinical population is available for study; during the period July 1, 1972 through February 28, 1973, 182 bronchoscopic procedures have been performed. These included 89 patients with lung cancer, 26 with lung abscesses, 13 chronic bronchitics, 23 pneumonia patients, and 31 others with various pulmonary disorders. The Pulmonary section at this hospital is comprised of 35 in-patient beds including 10 for the explicit purpose of acute and chronic convalescent respiratory care. For the purposes of this study all clinical work will be performed in conjunction with a pulmonary fellow and Dr. Goldstein. Patients will also be available from two affiliated institutions, George Washington University Hospital and the Washington Hospital Center.

The laboratory studies will be performed in the Pulmonary Immunology Research Laboratory of the Washington DC VAH under the direct supervision of Bernard W. Janicki, Ph.D. This unit is fully equipped for the studies planned in this proposal; the necessary radioisotope counting equipment and animal facilities are available in shared and common facilities.

11. Additional facilities required.

NONE

12. Biographical sketches of investigator(s) and other professional personnel (append).

A. Robert A. Goldstein, M.D.
B. Bernard W. Janicki, Ph.D.

13. Publications. (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See Sections 12 A and B.

SECTION 12A — CURRICULUM VITAE

NAME: Robert Arnold Goldstein

BORN:

RESIDENCE: REDACTED

MARITAL STATUS:

EDUCATION: Chester High School, Chester, Pa., R REDACTED

DEGREES: A. B. Brandeis University, Waltham, Mass.,

M. D. Jefferson Medical College, Phila. Pa. REDACTED

PROFESSIONAL EXPERIENCE: Philadelphia General Hospital, Phila. Pa
Internship, Mixed Medical, 1966-67

Veterans Administration Hospital, Wash. D. C.
Resident, Internal Medicine, 1967-69
Fellow, Pulmonary Disease, 1969-70

Tripler Army Medical Center, Honolulu, Hawaii
Ass't Chief, Pulmonary Disease Service, 1970-71
Chief, Pulmonary Disease Service, 1971-72

Veterans Administration Hospital, Wash, D. C.
Staff Physician, Pulmonary Disease Section and
Associate Chief, Pulmonary Immunology Research
Lab., 1972-

ACADEMIC APPOINTMENTS: University of Hawaii, School of Medicine
Honolulu, Hawaii, Ass't Clinical Professor of
Medicine, 1970-72

George Washington University School of Medicine
Washington, D. C, Ass't Professor of Medicine
and Staff Physician, 1972-

CONSULTANT: Queen's Hospital, Honolulu, Hawaii, 1970-72

National Institutes of Health, Pulmonary Medicine
Branch, Bethesda, Md. 1972-

Washington Hospital Center, Wash, D. C. 1973-

CERTIFICATION: National Board of Medical Examiners, 1967

American Board of Internal Medicine, 1972

LICENSE: Maryland, District of Columbia, Pennsylvania

MILITARY: Major, United States Army, 1970-72

100539609

MEMBER:

REDACTED

COMMITTEES: Program Chairman, Hawaii Thoracic Society, 1971-72

Program Committee, Scientific Assembly on Microbiology and Immunology, American Thoracic Society, 1973-

Medical Advisory Committee, American Lung Assoc. Southern Maryland, 1973-

RESEARCH INTERESTS: Pulmonary Disease and Immunology

PUBLICATIONS:

A. Journal Articles

1. Israel HL, Goldstein, RA: Metyrapone test in sarcoidosis. Amer Rev Resp Dis 98: 713-716, 1968.
2. Israel, HL, Goldstein, RA: Advances in sarcoidosis. Derm Digest 7:77-85, 1968
3. Goldstein, RA, Israel, HL: An assessment of serum protein electrophoresis in sarcoidosis. Amer J Med Sci 256: 302-313, 1968.
4. Goldstein, RA, Israel, HL, Rawnsley, HM: Effects of race and stage of disease on the serum immunolglobulin in sarcoidosis. J Amer Med Assoc. 208: 1153-1155, 1969
5. Israel, HL, Goldstein, RA: Frequency of disordered calcium metabolism in sarcoidosis. Postgrad Med J 46: 468-470, 1970.
6. Israel, HL, Goldstein, RA: Relationship of Kveim antigen reaction to lymph adenopathy: study of sarcoidosis and other diseases. New Eng J Med 284: 345-349, 1971.
7. Goldstein, RA, Israel HL, Becker, KL, Moore, CF: The infrequency of hyper calcemia in sarcoidosis Amer J Med 51: 21-30, 1971
8. Goldstein, RA, Becker, KL, Moore, CF; Serum urate in healthy men: intermittent elevations and seasonal effect. New Eng J Med 287: 649-650, 1972
9. Israel, HL, Goldstein, RA: Hepatic granulomatosis and sarcoidosis Annals Int Med (In press)
10. Goldstein, RA, Becker, KL, Israel, HL, Moore, CF: Urate metabolism in sarcoidosis. Arch Int Med (In press)

B. Abstracts

1. Janicki, BW, Goldstein, RA. Aron, SA: Immunoelectrophoretic studies of the precipitating antibody response in tuberculosis. Amer Rev Resp Dis 103: 894, 1971
2. Goldstein, RA, Yokoyama, M: Immunologic reactions in patients recovered from malaria. Fed Proc 31: 931, 1972
3. Goldstein, RA, Janicki, BW, Schultz, KE: Reactivity of lymphocytes from sarcoidosis patients to Kveim antigen in vitro. Clin Res 20: 378, 1972.
4. Goldstein, RA, Israel, HL, Janicki, BW, Yokoyama, M: Serum immunoglobulin D levels in sarcoidosis. Proceedings of the Sixth International Sarcoidosis Conference, Tokyo, 1972 (In press)
5. Goldstein, RA, Becker, KL, Israel, HL: The infrequency of hypercalciuria in sarcoidosis. Clin Res 21: 63, 1973.

1003539610

PRESENTATIONS

1. Fifth International Sarcoidosis Conference, Prague, 1969; British Thoracic and Tuberculosis Society, Cambridge, England, 1969: Total and ultra-filtrable serum calcium and magnesium in sarcoidosis.

2. Fifth International Sarcoidosis Conference, Prague, 1969: Serum Immuno-Globulins in sarcoidosis.

3. Eastern Section-American Federation for Clinical Research, Washington, D. C., 1969: Hypercalcemia and sarcoidosis.

4 District of Columiba Thoracic Society Symposium on Sarcoidosis, Washington, D. C., 1970: Immunology of sarcoidosis.

5. Southern Section-American Federation for Clinical Research, New Orleans, 1971: Relationship of Kveim reaction to lymphadenopathy in sarcoidosis.

6. American College of Physicians Regional Session, Honolulu,1971: Kveim antigen and sarcoidosis -- Clinical and Experimental Features.

7. American College of Physicians Regional Session, Honolulu, 1972: Newer immunological aspects of sarcoidosis.

SECTION 12B

CURRICULUM VITAE

BERNARD WILLIAM JANICKI, Ph.D.

PERSONAL

REDACTED

EDUCATION

University of Delaware

REDACTED University of Delaware

, The George Washington University

PROFESSIONAL EXPERIENCE

February 1954 to June 1955, University of Delaware, Research Assistant

September 1955 to present, Veterans Administration Hospital, Washington, D.C., Research Microbiologist, currently Chief of the Pulmonary Immunology Research Laboratory

PROFESSIONAL AFFILIATIONS

Scientific Organizations

REDACTED

Academic

The American University, Division of Sciences and Mathematics, Biology Department Thesis Advisory Committee, 1963

Graduate School, United States Department of Agriculture, Biological Sciences Department, Teaching Staff (Immunology), 1964

Georgetown University Schools of Medicine and Dentistry, Lecturer in Microbiology, 1965

University of Maryland, Lecturer in Microbiology, Department of Microbiology, 1969; Associate Member of the Graduate Faculty, 1970

10035396 12

Other

Consultant, National Institute of Allergy and Infectious Diseases, National Institutes of Health; serving as a member of the Tuberculosis Panel of the U.S.-Japan Cooperative Medical Science Program and Chairman of the Panel's Committee for a Collaborative Study of Tuberculin Antigens, 1969

RECOGNITIONS

Election to Beta Beta Beta, national honor society for Biological Sciences

Election to full membership in the Society of Sigma Xi

Listing in "American Men of Science"

RESEARCH INTERESTS

Basic aspects of host-parasite relationships with emphasis on acquired resistance of the host, especially immunity and delayed hypersensitivity

BIBLIOGRAPHY

BERNARD WILLIAM JANICKI, Ph.D.

THESES

Janicki, B.W.: Some aspects of the mechanism of penicillin resistance in pathogenic strains of Micrococcus pyogenes var. aureus. M.A. Thesis, University of Delaware, Newark, Delaware, 1955.

Janicki, B.W.: A study of the in vitro tuberculin lysis of leukocytes from tuberculin-hypersensitive guinea pigs. Ph.D. Thesis, The George Washington University, Washington, D.C., 1960.

PUBLICATIONS

1. Patnode, R.A., Hudgins, P.C. and Janicki, B.W.: Studies on the effect of Triton (WR-1339) on guinea pig tissues. I. Lipide chemistry of lung, liver, spleen, adrenals, and blood. J. Exp. Med. 107: 33-41, 1958.

2. Janicki, B.W.: Circulating levels of the "plasma factor" responsible for in vitro leukocyte cytolysis during sensitization of guinea pigs with Mycobacterium tuberculosis. Amer. Rev. Tuberc. 79: 244-245, 1959.

3. Janicki, B.W. and Patnode, R.A.: Enzymatic determination of in vitro lysis of leukocytes by tuberculin. Proc. Soc. Exp. Biol. & Med. 102: 311-314, 1959.

4. Janicki, B.W. and Patnode, R.A.: Studies on the effect of Triton (WR-1339) on the in vitro tuberculin lysis of leukocytes from tuberculin-hypersensitive guinea pigs. Tuberculology 19: 37-43, 1960.

5. Leahy, W.V.C., Janicki, B.W. and McNickle, T.F.: Effect of Triton WR-1339 on thyroid activity of normal guinea pigs. Amer. J. Physiol. 201: 827-829, 1961.

6. Janicki, B.W. and Patnode, R.A.: Increase in circulating lysozyme-like enzyme following sensitization of guinea pigs with Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. 83: 872-877, 1961.

7. Patnode, R.A. and Janicki, B.W.: Leukocytotoxic component of pine pollen. Effect on peripheral blood leukocytes of normal and tuberculin-hypersensitive guinea pigs. Amer. Rev. Resp. Dis. 83: 43-49, 1961.

8. Janicki, B.W. and Aron, S.A.: Effect of Triton WR-1339 on lipoproteins and lipoprotein lipase of guinea pig plasma. Proc. Soc. Exp. Biol. & Med. 109: 507-509, 1962.

9. Janicki, B.W., Aron, S.A. and Bernton, H.W.: Comparison of serological response to pine pollen and tubercle bacilli. Amer. Rev. Resp. Dis. 86: 385-390, 1962.

10. Janicki, B.W., Leahy, W.V.C., McNickle, T.F. and Aron, S.A.: Effect of Triton WR-1339 on absorption and fate of I-131 oleic acid in normal guinea pigs. Amer. J. Physiol. 202: 367-369, 1962.

11. Janicki, B.W. and Patnode, R.A.: Comparison of in vitro leukocytolysis produced with PPD and with fractions from culture filtrate of Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. 87: 748-752, 1963.

12. Miller, G.W. and Janicki, B.W.: Effect of Triton WR-1339 on malignant and normal cell cultures. Cancer Chemother. Rep. 39: 1-5, 1964.

13. Rose, M.J.,Jr., Aron, S.A. and Janicki, B.W.: Influence of non-ionic surfactants on bacteriophage infections. I. Effect of Triton WR-1339 on T2 coliphage infection. J. Bact. 87: 933-938, 1964.

14. Aron, S.A., Rose, M.J.,Jr. and Janicki, B.W.: Comparative serologic reactivity of I-131 and I-125 labeled human serum albumin preparations. Internat. Arch. Allergy 26: 255-263, 1965.

15. Jorolan, E.P. and Janicki, B.W.: Influence of some non-ionic surfactants on pancreatic lipase activity. Proc. Soc. Exp. Biol. & Med. 120: 313-316, 1965.

16. Rose, M.J.,Jr., Aron, S.A. and Janicki, B.W.: Effect of various non-ionic surfactants on growth of Escherichia coli. J. Bact. 91: 1863-1868, 1966.

17. Aron, S.A. and Janicki, B.W.: Influence of prior sensitization with mycobacteria on antibody response to protein antigen in Freund's adjuvants. Proc. Soc. Exp. Biol. & Med. 124: 518-524, 1967.

18. Janicki, B.W., Rose, M.J.,Jr. and Aron, S.A.: Influence of non-ionic surfactants on bacteriophage infections. II. Effect of Triton WR-1339 on members of the T2 coliphage serotype. In Chemistry, Physics and Application of Surface Active Substances 3: 417-425, edited by C. Paquot, Gordon and Breach Science Publishers, London, 1967.

19. Jorolan, E.P. and Janicki, B.W.: Mechanism of hyperlipemia induced by non-ionic surfactants. Progr. Biochem. Pharmacol. 2:308-314, 1967.

20. Janicki, B.W. and Aron, S.A.: Induction of tuberculin hypersensitivity and serologic unresponsiveness to tubercle bacilli in newborn and young guinea pigs. J. Immunol. 101:121-127, 1968.

21. Miller, G.W. and Janicki, B.W.: Further studies on the sellective cytotoxicity of Triton WR-1339. Cancer Chemother. Rep. 52:243-249, 1968.

22. Janicki, B.W., Schechter, G.P., and Schultz, K.E.: Cellular reactivity to tuberculin in immune and serologically-unresponsive guinea pigs. J. Immunol. 105:527-530, 1970.

23. Janicki, B.W., Chaparas, S.D., Daniel, T.M., Kubica, G.P., Wright, G.L., Jr., and Yee, G.S.: A reference system for antigens of Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. 104:602-604, 1971.

24. Goldstein, R.A., Janicki, B.W., and Schultz, K.E.: The Kveim controversy. Lancet, II, 1204-1205, 1971.

25. Janicki, B.W., Chaparas, S.D., Daniel, T.M., Kubica, G.P., Wright, G.L., Jr., and Yee, G.S.: An evaluation of a reference system for antigens of Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. 106:142-147, 1972.

26. Janicki, B.W., Aron, S.A. and Raychaudhuri, A.: Separation and isolation of mycobacterial antigens by continuous-flow electrophoresis. Amer. Rev. Resp. Dis. 106:779-783, 1972.

27. Janicki, B.W., Aron, S.A., Schechter, G.P., and McFarland, W.: Tuberculin antigens active in human lymphocyte blastogenesis. Proc. Soc. Exp. Biol. & Med. 141:809-813, 1972.

28. Barclay, W.R., Busey, W.M., Dalgard, D.W., Good, R.C., Janicki, B.W., Kasik, J.E., Ribi, E., Ulrich, C.E., and Wolinsky, E.: Protection of monkeys against airborne tuberculosis by aerosol vaccination with Bacillus Calmette-Guerin. Amer. Rev. Resp. Dis. 107:351-358, 1973.

29. Janicki, B.W., Aron, S.A., and Berson, A.S.: Technical factors affecting an immunoelectrophoretic reference system for analysis of mycobacterial antigens. Appl. Microbiol. 25:130-134, 1973.

30. Janicki, B.W., Good, R.C., Minden, P., Affronti, L.F., and Hymes, W.F.: Immune responses in rhesus monkeys after Bacillus Calmette-Guerin vaccination and aerosol challenge with Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. 107:359-366, 1973.

ABSTRACTS

1. Janicki, B.W. and Patnode, R.A.: The relationship of serum levels of lysozyme-like enzyme to sensitization of guinea pigs with M. tuberculosis. Amer. Rev. Tuberc. 79: 838, 1959.

2. Janicki, B.W.: Skin reactions and in vitro leukocytolysis with fractions from culture filtrate of M. tuberculosis in guinea pigs sensitized with heat-killed tubercle bacilli. Fed. Proc. 19: 211, 1960.

3. Janicki, B.W. and Patnode, R.A.: Comparison of in vitro leukocytolysis produced with PPD and with polysaccharide I fraction from culture filtrate of Mycobacterium tuberculosis. Bact. Proc. 87, 1960.

4. Janicki, B.W. and Aron, S.A.: Effect of some non-ionic detergents on enzymatic hydrolysis of coconut oil. Fed. Proc. 22: 575, 1963.

5. Rose, M.J.,Jr., Curtis, P.R., Aron, S.A. and Janicki, B.W.: Effect of Triton WR-1339 on phage infection of Escherichia coli. Bact. Proc. 1951, 1963.

6. Janicki, B.W., Rose, M.J.,Jr. and Aron, S.A.: Influence of a non-ionic surface active agent on coliphage infections. J. Gen. Microbiol. 37: v, 1964.

7. Jorolan, E.P. and Janicki, B.W.: Inhibition of the action of pancreatic lipase on coconut oil (Ediol) by some non-ionic surfactants. Amer. Chem. Soc. Abstr. 48C, 1964.

8. Jorolan, E.P., Janicki, B.W. and Sun, D.C.H.: Detergent influence on pancreatic lipase action. Fed. Proc. 24: 210, 1965.

9. Rose, M.J.,Jr., Aron, S.A. and Janicki, B.W.: Effect of various non-ionic surfactants on growth of Escherichia coli. Bact. Proc. 17, 1965.

10. Aron, S.A. and Janicki, B.W.: Characteristics of serological unresponsiveness to tubercle bacilli. Bact. Proc. 65, 1967.

11. Janicki, B.W. and Aron, S.A.: Induction of serologic unresponsiveness to tubercle bacilli. Fed. Proc. 26: 417, 1967.

12. Janicki, B.W. and Aron, S.A.: Immunologic unresponsiveness as a model for study of immunity in tuberculosis. Amer. Rev. Resp. Dis. 96: 161, 1967.

13. Janicki, B.W., Aron, S.A., Hawkins, J.E. and McClean, V.R: Experimental tuberculosis in serologically unresponsive guinea pigs. Bact. Proc. 65, 1967.

14. Janicki, B.W. and Aron, S.A.: Resistance to tuberculin shock in serologically unresponsive guinea pigs. Fed. Proc. 27: 494, 1968.

15. Janicki, B.W. and Aron, S.A.: Electrophoretic and immunoelectrophoretic separation of antigens in mycobacterial culture filtrates. Bact. Proc. 98, 1968.

16. Aron, S.A. and Janicki, B.W.: Immunoelectrophoretic analysis of guinea pig antibody response to mycobacterial antigens. Bact. Proc. 82, 1969.

17. Janicki, B.W., Raychaudhuri, A. and Aron, S.A.: Influence of incubation time on antigenic components in mycobacterial culture filtrates. Bact. Proc. 83, 1969.

18. Schultz, K.E., Roberson, B.S. and Janicki, B.W.: Comparison of dermal and cellular hypersensitivity to tuberculin in sensitized young guinea pigs. Bact. Proc. 69, 1969.

19. Aron, S.A., Raychaudhuri, A. and Janicki, B.W.: Separation of mycobacterial antigens by continuous-flow electrophoresis. Bact. Proc. 106, 1970.

20. Janicki, B.W., Aron, S.A., and Raychaudhuri, A.: Mycobacterial antigens active in tuberculin shock. Fed. Proc. 29: 702, 1970.

21. Janicki, B.W., Schechter, G.P. and Schultz, K.E.: Cellular reactivity to tuberculin in immune and unresponsive guinea pigs. Bact. Proc. 106, 1970.

22. Janicki, B.W., Aron, S.A., Schechter, G.P. and McFarland, W.: Tuberculin antigens active in human lymphocyte blastogenesis. Fed. Proc. 30: 465, 1971.

23. Janicki, B.W., Goldstein, R.A. and Aron, S.A.: Studies of the precipitating antibody response in tuberculosis. Amer. Rev. Resp. Dis. 103: 894, 1971.

24. Goldstein, R.A., Janicki, B.W. and Schultz, K.E.: Reactivity of lymphocytes from sarcoidosis patients to Kveim antigen in vitro. Clin Res. 20: 510, 1972.

25. Goldstein, R.A., Israel, H.L., Janicki, B.W., and Yokoyama, M.: Serum immunoglobulin D levels in sarcoidosis and tuberculosis. Clin. Res., 21:72, 1973.

14. First year budget

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Robert A. Goldstein, M.D.	15	REDACTED
Bernard W. Janicki, Ph.D.	30	
Pulmonary Fellow (to be named)	40	
Technical (annual salary plus fringe)		
Laboratory Technician, Immunology (to be named)	100	
Laboratory Technician, Histochemistry (to be named)	100	REDACTED
Sub-Total for A		

B. Consumable supplies (by major categories)	
Guinea pigs	500
Tissue culture glassware, media & reagents	1,200
Radioisotopes, solubilizer, fluor, & reagents	500
Disposable test tubes, pipettes, syringes, scintillation vials and other glassware	1,000
Chemicals, stains, buffers & reagents	200
Office supplies, reference materials, etc.	200
Sub-Total for B	3,600

C. Other expenses (itemize)	
Illustrations, film, photographs	400
Compensation for healthy volunteers	nil
Travel to scientific meetings for principal investigators	1,000
Sub-Total for C	1,400
Running Total of A + B + C	26,000

D. Permanent equipment (itemize)

NONE

Sub-Total for D	nil
E	3,900
Total request	29,900

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements.

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	3,800	2,400	nil	4,269	32,729
Year 3	REDACTED	4,000	2,500	nil	4,515	34,610

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Basic Institutional Support of Pulmonary Immunology Research Laboratory	Veterans Administration	62,463	7/1/72 - 6/30/73

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Basic Institutional Support of Pulmonary Immunology Research Laboratory	Veterans Administration	63,623	7/1/73 - 6/30/74

Robert A. Goldstein
Robert A. Goldstein, M.D.

Principal investigator s / Bernard W. Janicki
Bernard W. Janicki, Ph.D.

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Typed Name _____

Signature _____ Date 6/21/73

Telephone 202 (Area Code) 483-6666 (Number) 216 (Extension)

Checks payable to Medical Research Associates, Inc.

Mailing address for checks
Suite 511, 1133 15th Street, N.W.
Washington, D.C. 20005

Responsible officer of institution

Typed Name N. Marshall Meyers

Title Fiscal Officer

Signature N. Marshall Meyers Date 6/21/73

Telephone _____ (Area Code) R (Number) _____ (Extension)

10035396 20